

Four Different Cryoprotectors in Preservation of *Staphylococcus aureus*

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Preservation associated with the use of cryoprotectants has been one of the most used methods for the long-term storage of microorganisms, mainly because this technique can guarantee the conservation of the original characteristics of the standard strains. The present study aims to evaluate the effectiveness of Skim milk and glycerol as cryoprotectants in preserving the standard strain of *Staphylococcus aureus* ATCC 6538. Preservation was carried out in freezers at -20°C and -80°C , initially only between December from 2022 to March 2023. Viability and purity tests were performed after one day of freezing and after 3 months to evaluate the method's ability to maintain the survival and purity of the microorganism. After analyzing the results, the methods were efficient for preservation, confirming that the use of cryoprotective agents in the freezing of *Staphylococcus aureus* bacteria can be used in laboratory routine.

Keywords: Cryoprotective Agents. Standard Strain. Preservation. *Staphylococcus aureus*.

Introduction

The scientific community has been increasingly concerned with preserving and maintaining biological materials and living microorganisms in the laboratory [1]. This maintenance is essential due to the need to use organisms or specimens for scientific tests, for use in laboratory quality control, and to achieve maximum preservation of cell vitality and the number of viable cells [2]. Therefore, an acceptable preservation method is essential for the best results. This choice is made according to the phenotypic characteristics of the bacteria, the behavior of each species concerning preservation methods, the advantages and disadvantages of each technique, and the maximum time the strains can remain preserved. Therefore, the preservation time defines the classification of maintenance methods, which are short-term (continuous

priming), medium-term (preservation in mineral oil, preservation in sterilized water, freezing at -20°C and drying in silica gel, soil, and filter paper), or long-term (lyophilization and cryopreservation) [3].

However, medium (-20°C) and long-term freezing methods, if not carried out effectively according to the needs of each microorganism, can cause a deleterious effect, mainly on the cell membrane, and eventually cause cell death, because of the formation of crystals inside the cell. Therefore, it is essential to add cryoprotectants whose action protects against damage caused by freezing [4].

Thus, cryoprotective agents are substances that will be added to the means of suspension and will reduce the physical and chemical stress caused by freezing and thawing the cells, ensuring that the original characteristics of the standard strains are maintained. However, cryoprotectants must have low molecular weight, cellular toxicity, and high solubility in water to achieve this result. These agents include methanol, methyl acetamide, DMSO, glycerol, polysaccharides, mannitol, and skim milk [5,6].

The present work focused on the medium (-20°C) and long-term (-80°C) methods, comparing the efficiency and viability between four different types of cryoprotectants of the bacterial strains of *Staphylococcus aureus* ATCC 6538.

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Materials and Methods

A strain of the bacterium *Staphylococcus aureus* ATCC 6538 from the Microbiology laboratory was used by the SENAI Institute of Advanced Health Systems (ISI – SAS). These strains were reactivated from the replicate onto Tryptone Soybean Agar (TSA) medium followed by incubation for 24 hours at $37 \pm 1^\circ\text{C}$. After reactivation, the absence of contamination was confirmed from the macroscopic and microscopic visualization with the Gram stain and repeated in a selective medium of Salted Mannitol Agar. Subsequently, another replicate in TSA medium was followed by inoculating the strains for 24 hours at $37 \pm 1^\circ\text{C}$. Before proceeding to freeze, a last repeat was carried out with the addition in the media suspension, the following cryoprotectants: Skim milk 15% in Soy Tryptone Broth (TSB) plus glycerol 20%, skim milk 15% in water, 10% glycerol in TSB and 15% glycerol in TSB. All tests were repeated in 10 tubes and frozen at -20 and -80°C , followed by the viability test after one day of freezing to evaluate frozen strains' activity metabolism. The concentrations of the cryoprotectants were chosen based on the studies by Amorim and colleagues [4] and Becheleni and colleagues [7] with the necessary additions and alterations.

After three months of freezing, the strains were submitted to a new viability test with inoculation in TSA for 24 hours at $37 \pm 1^\circ\text{C}$, with subsequent macroscopic analysis, gram test, and catalase to confirm sample purity.

Results and Discussion

Using Skim milk and glycerol cryoprotectants in freezing and maintaining bacteria *Staphylococcus aureus* proved effective. The glycerol, by decrease the point of freezing and reducing the electrolytes in the non-frozen fraction, has an excellent protective effect becoming the cryoprotectant most used in freezing microorganisms [5]. Skim milk, in turn, acts efficiently in stabilizing the lipid bilayer during freezing and contributes to the stability of

cellular enzymes. It occurs due to the presence of lactose and calcium, milk components [5,8,9].

Another critical factor, in addition to the use of cryoprotectants, is to precede the freezing and refrigeration at temperatures between 2°C and 8°C to avoid the thermal shock that could occur due to temperature difference [8].

Conclusion

The four cryoprotectants were able to protect during freezing at -20 and -80°C for *Staphylococcus aureus* bacteria strains for initially three months. This result, therefore, reiterates the use of cryoprotective agents in order to preserve and conserve microorganisms without causing damage to the morphological, biochemical, and genetic structure of bacteria.

Further studies are necessary to continue evaluating long-term methods so that the results can be validated methods for prolonged use.

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